Complete Tumor Response Following Intratumoral ^{32}P BioSilicon on Human Hepatocellular and Pancreatic Carcinoma Xenografts in Nude Mice

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Abstract

Purpose: ^{32}P BioSilicon is a new, implantable, radiological medical device that comprises particles of highly pure silicon encapsulating ^{32}phosphorus (^{32}P) for the treatment of unresectable solid tumors. Prior to administration, the device particles are suspended in a formulant which provides an even suspension of the intended dose for implantation. The primary objective of this animal trial study was to investigate the effects of intratumoral injection of ^{32}P BioSilicon on human hepatocellular (HepG2) and pancreatic carcinoma (2119) xenografts implanted in nude mice (BALB/c). A secondary objective was the histopathologic examination of the tumor foci and surrounding tissue during the study.

Methods: Cultured human carcinoma cells (HepG2 and 2119) were injected s.c. into the gluteal region of nude mice. When the implanted tumors were ~1 cm in diameter, ^{32}P BioSilicon (0.5, 1.0, and 2.0 MBq) or formulant was injected into the tumors. Implanted tumor size was measured once a week for 10 weeks. At study termination, the tumor and surrounding normal tissue were collected and fixed in 10% formalin and processed for histopathologic analysis.

Results: ^{32}P BioSilicon produced a reduction in HepG2 tumor volume when compared with formulant control, and complete response was observed among tumors in the 1.0 and 2.0 MBq treatment groups after week 8. There was also significant reduction in 2119 tumor volume in all treated groups, with the complete response rate of 67% in the 2.0 MBq group.

Conclusion: ^{32}P BioSilicon suppressed the growth of both human hepatocellular and pancreatic carcinoma xenografts implanted in nude mice and complete responses were also observed in tumors at higher radiation doses.

In spite of advances in chemotherapy and radiation therapy, gains in the survival of common inoperable adult solid tumors have remained modest over the last 30 years (1). Surgical resection remains the only therapeutic modality that significantly prolongs survival, but with many solid tumors such as hepatocellular carcinoma and pancreatic carcinoma, resectability rates continue to be low and prognosis in unresectable patients remains poor.

Hepatocellular carcinoma is the third most common cause of cancer death worldwide, giving rise to >590,000 deaths per year (2). Although early lesions respond well to hepatic resection, liver transplantation, and some to percutaneous ablation or chemoembolization (3, 4), most patients with hepatocellular carcinoma still present with advanced disease, with only 10% to 20% of patients suitable for surgical intervention (5–7). External beam radiation is not useful for hepatocellular carcinoma because the sensitivity of the normal liver limits the dose that can be delivered, and chemotherapy is poorly efficacious (8). The median survival of inoperable patients remains about 3 months in places where the disease is endemic (9–11).

Pancreatic carcinoma similarly remains a major therapeutic challenge and tends to present in advanced, inoperable stages. Only around 15% to 20% of patients have resectable disease at presentation (12), but even in this group, the 5-year survival rate is only about 20% (13). In patients with unresectable pancreatic carcinoma and good performance status, treatment with chemoradiation results in a median survival of only 42 to 44 weeks (14, 15), although pain relief is obtained in 50% to 85% of the patients (16). Delivery of high-dose external radiation to the pancreas is limited by toxicity to the surrounding viscera, especially the small intestine.

The intratumoral administration of radiopharmaceuticals has the potential advantage of delivering the maximum amount of radioactivity to the tumor (17) with sharp dose fall-off to surrounding normal structures, thus limiting side effects. We postulate that delivery of high doses of radiation in such a localized setting will lead to significant tumor response with minimal toxicity and collateral acute radiation damage to other

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Table 1. Dosing schedules in the treatment animal groups

Group	Radiation dose (Gy)	^{32}P activity (MBq)	Injection volume (μL)	Group size (*n*)
Human hepatocellular carcinoma cell line HepG2				
1	formulant control	0	50	8
2	50	0.5	50	7
3	100	1.0	50	8
4	200	2.0	50	7
Human pancreatic carcinoma cell line 2119				
5	formulant control	0	50	8
6	50	0.5	50	10
7	100	1.0	50	10
8	200	2.0	50	9

tissues if the source of radiation remains locked *in situ* with minimal systemic distribution.

Phosphorus 32 comes close to being the ideal unsealed therapeutic radionuclide (18). It is a pure β-particle emitter with a physical half-life of 14.3 days. The maximum range in tissue of the ^{32}P β-particle emission is about 7.6 mm. Colloidal ^{32}P has been used for the treatment of intracavitary malignancies. The retention of radioactivity of colloidal ^{32}P at the site of a solid tumor required the infusion of macroaggregated albumin (19). An alternative method to aid radioactivity retention at the tumor site has been to use labeled, nontoxic and undegradable microcarriers such as phosphorus-32 glass microspheres (20).

BioSilicon (21), recently developed by pSiMedica (Malvern Hills, United Kingdom), has characteristics that make it potentially ideal for use as a vehicle for intratumoral delivery. BioSilicon refers to etched forms of semiconducting silicon that have been porosified with electrochemical techniques in hydrofluoric acid–based solutions (22). This new biomaterial can be micronized and classified into particles of tunable size in order to optimize localization within the tumor. In addition, there is increasing *in vitro* and *in vivo* evidence of its biodegradability and tissue compatibility (21, 23–25). In our previous mice study, intratumoral injection of BioSilicon and formulant-only had not shown any significant toxic effects on the animals when compared with the no-treatment control group.[7]

The objective of this study was to study the effects of intratumoral ^{32}P BioSilicon on the growth of solid tumor xenografts in an *in vivo* tumor model. S.c. implants of hepatocellular and pancreatic carcinoma xenografts in nude mice (BALB/c) were subjected to brachytherapy from radioactive ^{32}P BioSilicon devices which were comprised of 20 μm polysilicon powder containing phosphorus 32 as the radionuclide.

Materials and Methods

^{32}P BioSilicon. The test substance, ^{32}P BioSilicon, an active implantable, radiological medical device with phosphorus 32 activity, was created by thermal neutron capture of highly phosphorus-doped silicon within a nuclear reactor, which transmutes the natural phosphorus (^{31}P) in the silicon lattice of BioSilicon to the β-emitting radionuclide ^{32}P. This is a similar process to that used by the semiconductor industry to dope silicon ingots and wafers with phosphorus, where the silicon 30 isotope is transmuted to phosphorus 31 (26). The activity of the product is 75 MBq nominal activity/100 mg BioSilicon (at reference date). The ^{32}P BioSilicon is then filled and sterilized at the radiopharmacy of AEA Technology QSA (Braunschweig, Germany). Just before injection, it was suspended in an aqueous suspending solution containing microcrystalline cellulose and sodium carboxymethylcellulose.

Animals and tumors. Eight-week-old male nude mice (BALB/c; The Animal Resources Centre, Murdoch, Western Australia)were housed in pathogen-free conditions conforming with the guidelines of the National Advisory Committee for Laboratory Animal Research of Singapore.

Human hepatocellular carcinoma cell line HepG2 and human pancreatic carcinoma cell line 2119 were obtained from the American Type Culture Collection (Manassas, VA) and cultured in MEM with 10% FCS in 5% CO_2 incubator. Cells (5×10^6) suspended in 100 μL of HBSS were injected s.c. into the right gluteal region of the nude mice.

Injection of ^{32}P BioSilicon in transplanted tumors. On day 15 after implantation of tumor cells, when the tumors were about 1 cm in diameter, ^{32}P BioSilicon was injected in the center of the tumors at the depth of about 4 mm.

Animal groups. Animals were divided into control and ^{32}P BioSilicon groups. The control group was injected with formulant only. ^{32}P BioSilicon was injected at three different activities of 0.5, 1.0, and 2.0 MBq, corresponding to absorbed tumor doses of 50, 100, and 200 Gy, respectively, as listed in Table 1.

Measurement of tumor volume. The sizes of the implanted tumors were estimated every 7 days. The largest and smallest diameters were measured by a vernier caliper and tumor volume was estimated according the formula: $V = 1/2\ ab^2$, where a and b are the largest and smallest tumor diameters, respectively, and V is the tumor volume in milliliters. The effect of ^{32}P BioSilicon was assessed by comparing the tumor volumes of treatment groups to the control group at each week, up to week 10. The means and SD of the tumor volumes expressed as a percentage change from day 0 were calculated for each dose group at each time point. The SE was calculated as the SD divided by the square root of number of animals in each group.

Definition of complete response. When the tumors become undetectable after implantation of ^{32}P BioSilicon, tumor volumetric

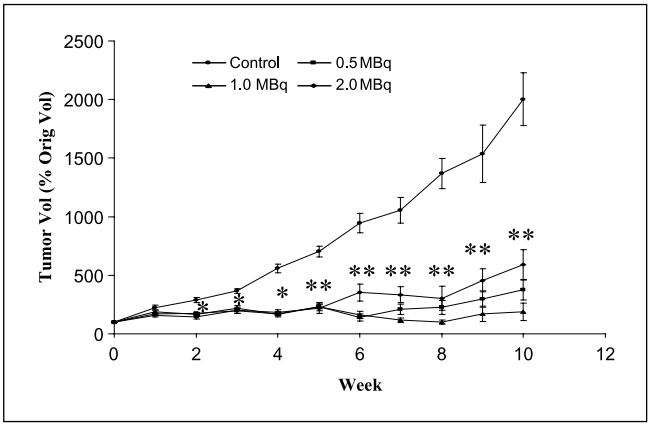

Fig. 1. Tumor volumetric assessment of the effect of BioSilicon on pancreatic carcinoma cell line 2119 xenografts in nude mice. The average tumor volume for each dose group is expressed as a percentage of change in tumor volume compared with week 0. Points, means; bars, ± SE. The average tumor volumes of formulant control, 0.5, 1.0, and 2.0 MBq groups at week 0 are 88.9, 78.4, 63.5, and 65.3 mm^3, respectively. Comparisons between control and ^{32}P BioSilicon treatment groups by ANOVA. *, $P < 0.05$; **, $P < 0.005$.

[7] Unpublished data.

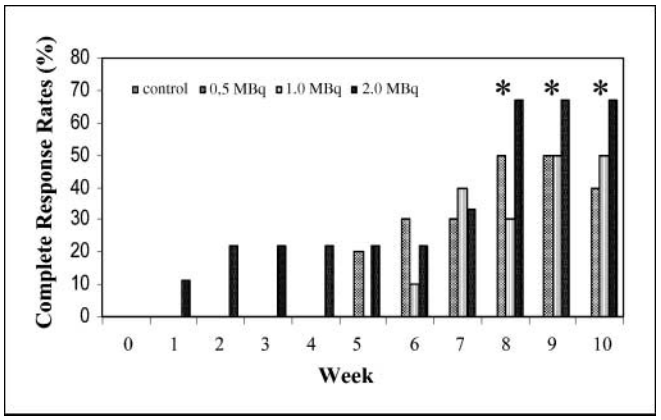

Fig. 2. Tumor complete responses in pancreatic 2119 xenografts. The complete response rates (expressed as a percentage) of each group at each time point. Comparisons between control and ^{32}P BioSilicon treatment groups by χ^2 test. *, $P < 0.05$.

measurements could not be made and histologic examination of implantation sites shows no viable tumor tissue.

Histologic studies. On days 10, 20, and 30 after ^{32}P BioSilicon injection, the animals were killed by an overdose of pentobarbitone at 50 mg/kg of the sedated animal. The tumor and surrounding normal tissue were collected and fixed in 10% formalin. The samples were kept in −80°C for half a year and when there was no longer any detectable radioactivity, the sample was then processed for sectioning and staining with H&E. This is in accordance with the rules of radiation safety of the institution.

Statistical analysis. One-way ANOVA was used for analysis of significance of the differences of relative tumor volume changes between control and treatment groups and post hoc tests for analysis of the differences between any two groups. Significance of the differences of complete response rates of each group at each time point were analyzed by χ^2 test. SPSS was used for these statistical analyses. $P < 0.05$ was considered to be statistically significant. The animal studies were approved by the Institutional Animal Care and Use Committee of the Singapore General Hospital.

Results

Figure 1 shows the percentage of change in tumor volume of pancreatic carcinoma (2119) xenografts, compared with the tumor volume at day 0 (the day of ^{32}P BioSilicon injection). All three doses of ^{32}P BioSilicon resulted in statistically smaller tumors at each of the time points assessed, beginning in the 2nd week postinjection, up to week 10, when the study was terminated. However, no significant dose-response relationship was observed. When analyzed by ANOVA, the differences between control and treatment groups are very significant ($P < 0.05$ to $P < 0.0001$), whereas the differences between the three dose groups are not significant ($P > 0.5$ to $P > 0.9$). We defined tumor complete response as when no tumor was detectable, tumor volumetric measurements could not be made and histologic examination of explanted tissue did not show evidence of tumor. The complete response rates in each animal group at each time point are summarized in Fig. 2. Except for the control group, tumor complete response was seen in all the treatment animal groups, and the differences of complete response rates between treatment groups and control group were shown to be significant at weeks 8, 9, and 10 ($P < 0.05$). Thus, whereas ^{32}P BioSilicon implantation resulted in complete tumor responses, this was independent of radiation dose in the dosage range of 0.5 to 2.0 MBq.

Similar effects were observed for the hepatocellular carcinoma (HepG2) xenografts and ^{32}P BioSilicon also resulted in a statistically significant reduction in the change in tumor volumes across the three different activities when compared with controls (Fig. 3). Tumor complete responses were also observed in treatment groups (Fig. 4).

Complete responses were confirmed by histopathologic examination which showed no viable tumor tissue in the specimens (Fig. 5). In tumors which did not undergo a complete response, histologic examination shown that necrosis was the mode of cell death (Fig. 6). In this study, the amount of radioactivity that localized to the tumor site was not measured, partly because of difficulties visualizing bremsstrahlung in small animals such as mice. In one of our other studies, ^{32}P BioSilicon was injected into the liver of a larger animal model (pig) and autoradiographs of the pig livers showed discrete and localized uptake of ^{32}P BioSilicon to the injected site only.[7]

Discussion

The main hurdle to efficacious radiation therapy in solid tumors is the therapeutic ratio applicable to that malignancy, i.e., the relationship between the tumor lethal dose and the tolerance of normal tissues in the vicinity of the tumor. Brachytherapy techniques extend the use of radiation therapy by changing the therapeutic ratio in favor of increased radiation to the tumor. The application of brachytherapy to deep-seated organ sites however remains limited by practical considerations of delivery of the radiation source.

Chemotherapy is poorly efficacious in most inoperable solid tumors such as hepatocellular carcinomas (8), and similarly, chemoradiation in pancreatic carcinoma (14, 27). Intratumoral brachytherapy thus becomes especially attractive in such cases if lethal dose (and resulting tumor regression) may be achieved with minimal damage to other tissues both adjacent and

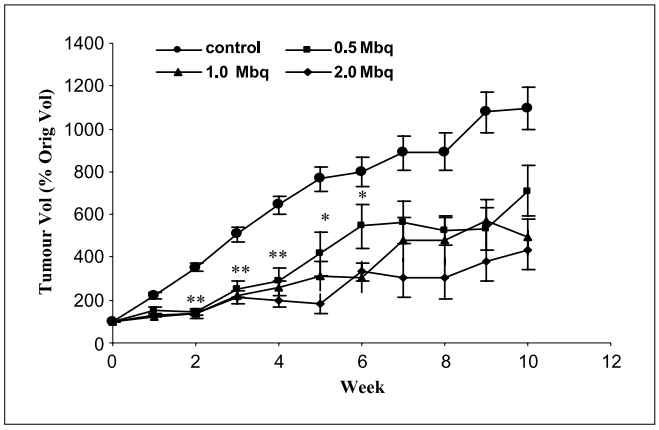

Fig. 3. Tumor volumetric assessment of the effect of BioSilicon on hepatocellular carcinoma cell line HepG2 xenografts in nude mice. The average tumor volume for each dose group is expressed as a percentage of change in tumor volume compared with week 0. Points, means; bars, ± SE. The average tumor volumes of formulant control, 0.5, 1.0, and 2.0 MBq groups at week 0 are 134.2, 105.2, 83.5, and 121.3 mm^3, respectively. Comparisons between control and ^{32}P BioSilicon treatment groups by ANOVA. *, $P < 0.05$; **, $P < 0.005$.

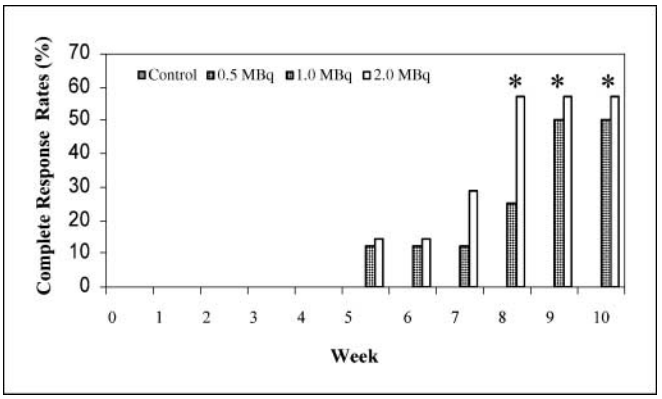

Fig. 4. Tumor complete responses in the HepG2 (hepatocellular carcinoma) xenografts. The complete response rates (expressed as a percentage) of each group at each time point. Comparisons between control and ^{32}P BioSilicon treatment groups by χ^2 test. *, $P < 0.05$.

systemic. Essential to successful intratumoral brachytherapy is therefore the need for the radiation source to remain localized at the tumor site and with minimal systemic distribution. The material used as vehicle to convey the source of radiation to the tumor as well as the means of ensuring that the source of radiation remains locked *in situ* thus assumes great importance.

There have been few animal studies (20, 28–30) and clinical trials (15, 19, 31, 32) on intratumoral brachytherapy in hepatocellular carcinoma and pancreatic carcinoma. These have either used glass microspheres as a vehicle or infusional maccroaggregated albumin as a means to aid radioactivity retention at the tumor site. Although significant tumor regression has been recorded using both methods, in the clinical trial using ^{90}Y-microspheres, significant leakage of radioactivity was documented, with left lung radioactivity in 6 of 11 patients and intestinal activity in 4 patients (31).

The procedures involved in the use of infusional macroaggregated albumin to aid ^{32}P radioactivity retention at the tumor site were complex but the results were reported to be efficacious. In the trial by Firusian et al. (32), only 3 of the 17 patients had hepatocellular carcinoma but good responses were recorded in all three cases. A clinical study using infusional macroaggregated albumin in combination with external beam radiation and chemotherapy in unresectable pancreatic adenocarcinoma similarly reported good response with complete remission (19, 33).

The majority of patients with hepatocellular carcinoma and pancreatic adenocarcinoma are inoperable at the time of diagnosis and there is pressing need for efficacious therapy in these patients. The number of patients with hepatocellular carcinoma in particular seems to be increasing in some areas of the developed world as a result of increasing prevalence of viral hepatitis C (34, 35). This current study using a single intratumoral injection of ^{32}P BioSilicon showed significant tumor reduction in hepatocellular carcinoma and pancreatic carcinoma xenografts in nude mice and with complete response at the late stage of the treatment period (Figs. 2 and 4). In addition to its biodegradability and compatibility with animal tissue (21, 22), this novel brachytherapy device has shown excellent efficacy with complete tumor regression (Figs. 2 and 4; $P < 0.05$). In this respect, it seems to be a more efficacious delivery vehicle for ^{32}P brachytherapy when compared with other materials tested (20, 28, 29). However, no significant dose-response relationship was observed (Figs. 1–4; $P > 0.2$ to $P > 0.7$) and this suggests that optimal radiation doses in this model may have been achieved. From tumor volumetric analysis (Fig. 1), the lowest dose of 0.5 MBq used almost reached the maximum therapeutic effect. We postulate that the dose of 0.5 MBq of ^{32}P BioSilicon is above the linear range of the dose-response curve (on top of the S-shaped curve). Further increase of dosage could not enhance the effects proportionally. Good localization of ^{32}P

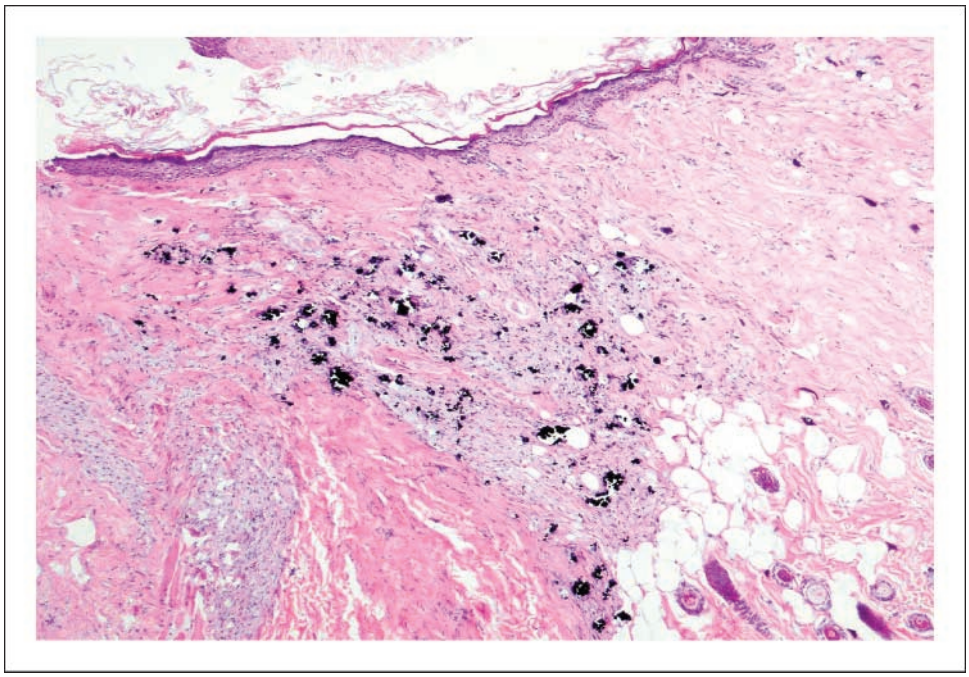

Fig. 5. No viable tumor was seen in this specimen showing skin, a focus of scarring and pigmented material, taken from xenograft of 2119 injected with 2.0 MBq of BioSilicon.

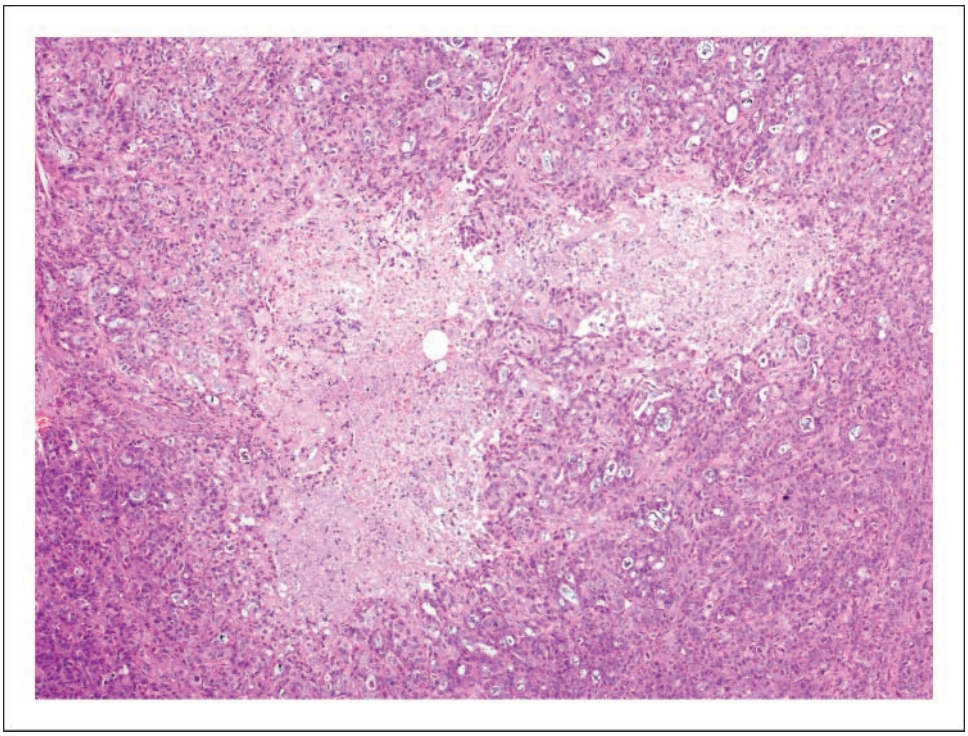

Fig. 6. Tumor nodule from 2119 xenograft injected with 0.5 MBq of BioSilicon showing focus of tumor necrosis.

BioSilicon to the injected site with only minimal systemic distribution was also shown in our study in a large animal model.[7]

The data from both this study and other previous studies suggests that intratumoral radiation therapy may offer patients with unresectable hepatocellular carcinoma and pancreatic tumors efficacious therapy and a chance for prolonged survival. The introduction of ^{32}P BioSilicon in tumors is also a simpler procedure compared with infusional macroaggregated albumin and supplies a high level of radiation in a small amount of excipient, and this ease of application may potentially also translate to efficient administration to patients. The safety and efficacy of ^{32}P BioSilicon in inoperable hepatocellular carcinoma is currently being investigated in an early phase clinical trial.

Acknowledgments

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